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Research paper

Validation of a digital PCR method for quantification of DNA copy number concentrations by using a certified reference material

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Abstract

Digital PCR has become the emerging technique for the sequence-specific detection and quantification of nucleic acids for various applications. During the past years, numerous reports on the development of new digital PCR methods have been published. Maturation of these developments into reliable analytical methods suitable for diagnostic or other routine testing purposes requires their validation for the intended use.

Here, the results of an in-house validation of a droplet digital PCR method are presented. This method is intended for the quantification of the absolute copy number concentration of a purified linearized plasmid in solution with a nucleic acid background. It has been investigated which factors within the measurement process have a significant effect on the measurement results, and the contribution to the overall measurement uncertainty has been estimated. A

comprehensive overview is provided on all the aspects that should be investigated when performing an in-house method validation of a digital PCR method.

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Keywords

Digital PCR; Method validation; Measurement uncertainty; Certified reference materials

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